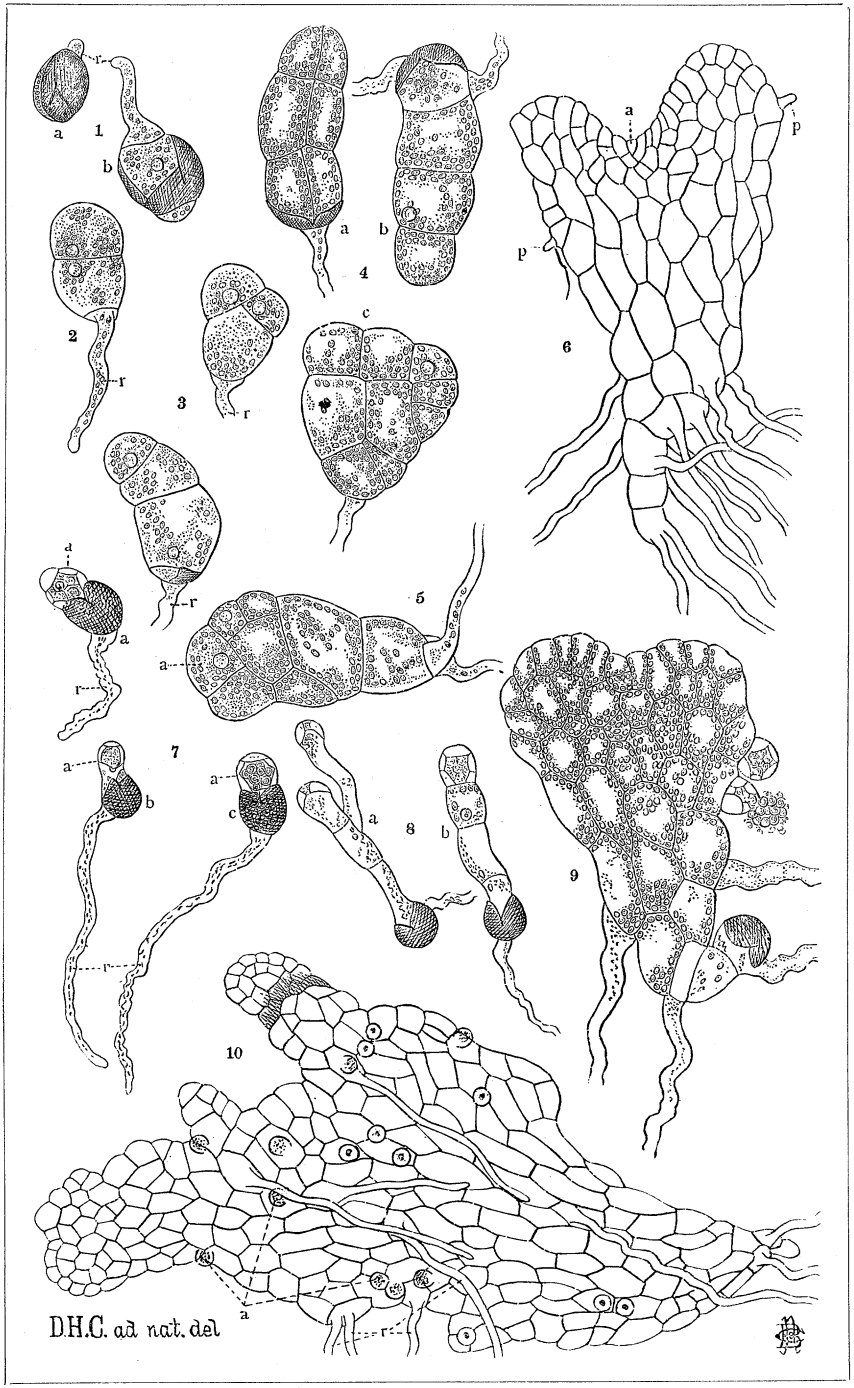

CAMPBELL ON PROTHALLIA.

The Development of the Prothallia of Ferns.[1]

BY DOUGLAS H. CAMPBELL.

(WITH PLATE IX.)

In the following paper an attempt has been made to present in brief form the results of a series of experiments upon the germination of the spores, and the development of the prothallia of some of the common ferns.

This subject was chosen for two reasons: first, because in the course of the experiments attention was called to several facts that do not seem to be generally known; and secondly, if possible, to arouse the interest of students of botany in a line of work that does not appear to receive the attention that it deserves.

The importance of the study of the development for a full understanding of any group of organic bodies is fully admitted, but there seems to be a prevalent idea that such study must necessarily be confined to the experienced and thoroughly trained biologist, whereas frequently many of the most important facts of development can be verified by any student of ordinary ability who knows how to handle a microscope.

The plants under consideration offer perhaps the best illustration of the point in question. Complex as is the structure of the mature fern, it starts from a single cell, the spore, and the earlier steps in the development of the plant from this cell can be traced with little difficulty. Of course the development of the sexual organs and the growth of the fern itself from the fertilized germ-cell is a much more difficult matter, but up to this point no difficulty is experienced.

There is no part of the country where some ferns do not grow, and the spores may either be sown at once or kept for future use, as they retain their vitality for a long time. They germinate quickly, and with a little care can be kept in a healthy condition and the successive steps in the development of the prothallium followed.

The first fern investigated by me was *Onoclea Struthiopteris*. After vain attempts to procure a sufficient number of fern-prothallia from green-houses, for study, and supposing that the germination of the spores was a long and difficult process, I determined to make some experiments. A quantity of the spores were gathered and sown without much hope of success, but in a few days undoubted signs of germination were evident, and soon

[1] Read before the American Association for the Advancement of Science, August, 1885.

a large number of healthy young prothallia was the result. Encouraged by this success, *O. sensibilis* was tried with similar results. Lack of time prevented following out the complete development of the prothallium, but subsequently both forms were grown and produced young plants. Other species, upon which more or less complete observations have been made, are *Asplenium Filix-fœmina*, *Aspidium spinulosum*, *A. acrostichoides*, *Adiantum pedatum*, *Cystopteris fragilis*, and *Woodsia Ilvensis*. Of these *Aspidium spinulosum* is frequently found growing naturally, as is also *Cystopteris*, but whatever observations were made upon the others were from artificial cultures. Spores were sown under various conditions, but the best results were obtained by simply sowing upon fine earth. The earliest stages can be more conveniently observed by sowing in water, but prothallia so grown do not maintain a healthy growth, and so far as my observations go, no sexual organs were developed upon them. Other cultures were made upon rotten wood, upon which they are frequently found naturally, and upon porous pottery, but in neither case was the result as good as when grown upon damp earth. In order to maintain an even degree of moisture, it was found best to keep the prothallia covered with a bell-jar, or some similar contrivance, as evaporation is too rapid when they are exposed to the air. Excess of moisture, especially after the prothallia are at all advanced, must be guarded against, as they will decay if kept too wet. The ground should be kept damp, but *not muddy*. Spores may be started at any time, but the best results are obtained by sowing in warm weather, or at least when they can be kept at a tolerably uniform temperature. In winter, even with artificial heat, growth almost ceases, the deficient light seeming to affect them unfavorably, as well as the lower temperature. Probably in a green-house, where the fluctuations of temperature are less marked, this would be less apparent.

By following the directions given, no trouble should be experienced in growing any of our common ferns.

The spores of ferns are protected by a thick brown exospore, usually marked by prominent thickenings or ridges. In many cases this exospore remains attached to the spore for a long time after germination. but in the genus *Onoclea*, it frequently becomes thrown off, being readily detached even when the spores are not fully mature. In this genus there is in addition to the exospore a second coat, which must be ruptured before the endospore proper can protrude. In ferns of other genera examined the exospore adhered so firmly to the spore as to make it impossible to determine whether this third coat was present or not.

The contents of the spore consist of dense protoplasm containing a nearly central nucleus, and numerous fine granules of chlorophyll. Oil is present, usually uniformly distributed, but sometimes collecting in large drops.

Ordinarily the first step in the active growth is an elongation of the spore, one end becoming nearly transparent. This transparent end elongates still farther and very soon is shut off by a septum approximately at right angles to the longer axis of the spore. Two cells are thus formed, a small transparent one, the first root-hair, and a larger one containing abundant chlorophyll. The root-hair contains only a few very small granules of chlorophyll, and these finally seem to disappear entirely. The walls of the root-hair frequently show a decided brown color, which is sometimes developed very early. This is noticeably the case in *Onoclea sensibilis;* in *Asplenium filix fœmina*, on the contrary, the color is scarcely apparent even in the older root-hairs. Abnormal cases occur where no root-hair is formed. The larger of the two cells first formed also elongates and very soon undergoes further division (Figs. 1–3). In the great majority the first division, and usually several successive septa, are parallel to the primary septum, thus giving rise to a filament, or single row of cells. The prothallium may never pass beyond this condition, especially if it is grown in water, but ordinarily, sooner or later, the terminal cell becomes divided by a septum nearly at right angles to the first ones (Fig. 4). Sometimes, again, the first wall in the body of the spore may be at right angles to the primary septum, and the prothallium never passes through the filamentous stage—this, however, appears to be exceptional (Fig. 4, a).

While the division of the cells is progressing, the chlorophyll granules which were small and crowded at first, become much larger and more widely separated. As the prothallium grows, they enlarge and divide, all of those to be found in the complete prothallium apparently arising from the growth and division of those that occur in the spore before germination.

After a varying number of divisions, one of the terminal cells assumes the lead and becomes a triangular apical cell, dividing by septa directed alternately right and left (Fig. 5–6, a). Occasionally two successive septa will be formed on the same side. As a result of the repeated divisions of this apical cell, the end of the prothallium becomes rapidly broader, but for a time the deep sinus in front, so characteristic of the prothallium, is not present. This arises as follows: each segment is first divided

by a septum perpendicular to the long axis of the prothallium; the outer of the two cells thus formed, or marginal cell, becomes next divided by a wall perpendicular to the first formed. These marginal cells lengthen, and the inner cell of the segment divides still further by walls parallel to the first. The segment thus grows faster in length than in breadth, and is rapidly pushed out beyond the apical cell, which thus gradually comes to lie at the bottom of an indentation or sinus in the front of the prothallium, and gives it the heart shape or kidney shape that the larger prothallia usually have. After a time, varying extremely even in the same species, the apical cell is divided into two cells by a vertical wall perpendicular to the long axis of the prothallium; the outer cell is next divided by a wall at right angles to the first, and from this time in the growth can no longer be traced back to one cell.

About this time, or sometimes before the obliteration of the apical cell, the cells in its vicinity, which had hitherto only divided in two planes, so as to form a single layer of cells, are now divided by walls parallel to the surface of the prothallium, forming the beginning of the cushion of tissue that occupies the base of the notch in the front of the prothallium; where this is begun early, the subsequent growth of the prothallium results in the formation of a thickened rib running through the middle for nearly its whole length.

The prothallium is fastened to the ground by numerous root-hairs that arise principally from the cells of the lower part, as small papillæ, that are soon shut off by a septum. Like the first one formed they contain little or no chlorophyll, and in most species soon acquire brown walls. They probably serve to some extent as absorbents as well as to fasten the plant.

In many species, e. g. *Aspidium spinulosum*, *Cystopteris fragilis*, *Onoclea sensibilis*, there are developed small papillæ from the marginal cells (Fig. 6. p.); whether these have any special function is extremely doubtful. They are entirely wanting in *Asplenium filix-fœmina*, and *Onoclea Struthiopteris*.

While the foregoing statements are true for the majority of cases, it must not be inferred that they are without exceptions; indeed, probably none of them are absolutely inflexible, and except in the earlier stages they are true only of the larger prothallia, which are, in most cases observed, female.

The subject of diœcism in ferns is not, apparently generally understood; in fact, as far as I have been able to ascertain, while the formation of prothallia bearing only antheridia has been ob-

served, but apparently regarded as exceptional, the formation of strictly female prothallia has been overlooked. Of the ferns mentioned in this paper, three have been traced out with care, from the spore to the formation of the embryo, viz: *Onoclea Struthiopteris*, *O. sensibilis*, and *Asplenium filix-fœmina*, and in every case it was exceptional to find both sexual organs on the same prothallium. Asplenium offered the greatest number of exceptions, but even here it was rare. *Aspidium spinulosum*, to judge from naturally grown specimens, is monœcious, no undoubted male prothallia being found, and perfect antheridia occurring on the large prothallia. *Cystopteris fragilis* was found with two forms; small male prothallia, and large hermaphrodite ones. In regard to other forms, observations were too incomplete to warrant any statements on the subject.

The male prothallia of all the forms observed are, as a rule, very much smaller than the female, and frequently of very irregular shapes (Figs. 8, 9, 10). Either no definite apical cell is formed, or it is early lost. Where the spores are sown thickly it is not uncommon to find the prothallium reduced to a single row of cells terminated by an antheridium, other antheridia being formed laterally in some cases, in others not.

The simplest form observed was that of *Asplenium filix-fœmina*, where a number of prothallia were formed whose vegetative portion was reduced to a single cell besides the root-hair (Fig. 7). A single antheridium was formed in each, and perfect antherozoids.

Under favorable circumstances the spores germinate in from three to five days, but in cold weather, or with imperfect spores, the time was indefinitely lengthened. The first antheridia were usually mature in about five weeks from the first signs of germination, but in one case, Asplenium, ripe antheridia were formed in a little more than three weeks from the sowing of the spores. Spores sown August 31 produced ripe antheridia on September 23. The prothallia in this case were reduced to a very few cells. Spores of *Onoclea Struthiopteris*, sown on February 25, 1884, began to grow upon March 3d, produced the first ripe antheridia April 9th, and the first mature archegonium, April 30th.

As an instance of the vitality of the spores, one example will suffice. Specimens of *Woodsia Ilvensis* were collected upon Lake Superior in July, 1883, the fronds were dried for the herbarium, and laid aside. In October, 1884, a number of the spores were placed in water, and in about a week began to grow.

Under proper conditions the prothallia will live for compara-

tively long time. Cultures were made of *Asplenium* and *Onoclea Struthiopteris* in 1884, from spores sown August 31. They grew rapidly and soon developed quantities of antheridia, but in the case of *Asplenium, no archegonia.* As cold weather came on, although the prothallia were kept in the house, growth ceased almost entirely, but was resumed in the spring, the prothallia appearing perfectly healthy. Large numbers then developed archegonia, and subsequently young plants, but some of them are still (August 14) perfectly vigorous, and growing.

The older male prothallia assume very irregular forms, reminding one somewhat of the prothallia of *Equiseta*, but the female retain nearly the same form as they have when young although they may be slightly irregular.

The sexual organs continue to form as the prothallia grow, so that in the older ones the number is very large. A female prothallium of Asplenium, about one cm. in diameter, examined August 12, had over one hundred and twenty-five archegonia, but no antheridia. One of the archegonia had been recently fertilized, but the remainder were abortive. It sometimes happens that several archegonia will be impregnated, but only one embryo apparently ever develops perfectly.

In the older prothallia of *Onoclea Struthiopteris*, especially early in the spring, large quantities of starch were observed.

EXPLANATION OF PLATE IX.—Figs. 1–5, successive stages in germination of the spore of *Onoclea sensibilis*. × 150. Fig. 6, an older prothallium of the same, showing the apical cell (a), and marginal papillæ (p). × 80. Fig. 7, very small male prothallia of *Asplenium filix-fœmina* antheridia (a). × 150. Figs. 8–9, large male prothallia of the same. × 150. Fig. 10, a male prothallium, about three months old, of *Onoclea Struthiopteris*. × 60. Fig. 11, mature prothallium of *Cystopteris fragilis*. × 5.

On the Appearance of the Relation of Ovary and Perianth in the Development of Dicotyledons.[1]

BY JOHN M. COULTER.

Two years ago I read before this section a paper upon the "Development of the Dandelion," an organogenic study, in which it incidentally appeared that the first character to show itself was that of the inferior ovary. Since then this hint has been some-

[1] Read before the American Association for the Advancement of Science, August, 1885.